AMENDMENTS IN THE SPECIFICATION

Please amend the specification as follows, to correct typographical errors.

Page 1, lines 12-14 Please replace the "Technical Field" paragraph with the following paragraph:

Technical Field

Embodiments of the ~~resent~~ recent invention provide systems and methods for utilizing gene expression analysis for characterizing a biological condition or agent.

Page 21, first full paragraph Please replace the first full paragraph on p. 21 (lines 4-23) with the following paragraph:

We have exemplified the use of selected panels of constituents corresponding to gene loci from which quantitative gene expression is determined by, for example, quantitatively measuring the transcribed RNA in a sample of a subject, for applications that include: (a) measurement of therapeutic efficacy of natural or synthetic compositions or stimuli that may be formulated individually or in combinations or mixtures for a range of targeted physiological conditions; (b) predictions of toxicological effects and dose effectiveness of a composition or mixture of compositions for an individual or in a population; (c) determining how two or more different agents administered in a single treatment might interact so as to detect any of synergistic, additive, negative, neutral ~~of~~ or toxic activity (d) performing pre-clinical and clinical trials by providing new criteria for pre-selecting subjects according to informative profile data sets for

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revealing disease status and conducting preliminary dosage studies for these patients prior to conducting phase 1 or 2 trials. Gene expression profiling may be used to reduce the cost of phase 3 clinical trials and may be used beyond phase 3 trials; (e) labeling for approved drugs; (f) selection of suitable medication in a class of medications for a particular patient that is directed to their unique physiology; (g) diagnosing or determining a prognosis of a medical condition or an infection which may precede onset of symptoms or alternatively diagnosing adverse side effects associated with administration of a therapeutic agent; (h) managing the health care of a patient; and (i) quality control for different batches of an agent or a mixture of agents.

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Page 25, only full paragraph Please replace the "Gene Expression" paragraph on p. 25 with the following:

Gene Expression

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For measuring the amount of a particular RNA in a sample, we have used methods known to one of ordinary skill in the art to extract and quantify transcribed RNA from a sample with respect to a constituent of a selected panel (See detailed protocols below.) Briefly, RNA is extracted from a sample such as a tissue, body fluid (see Example 11 below), or culture medium in which a population of a subject might be growing. For example, cells may be lysed and RNA eluted in a suitable solution in which to conduct a DNAse reaction. First strand synthesis (see Example 10 below) may then be performed using a reverse transcriptase. Gene amplification, more specifically quantitative PCR assays, can then be conducted and the gene of interest size calibrated against a marker such as 18S rRNA (Hirayama et al., Blood 92, 1998: 46-52). Samples are measured in multiple duplicates, for example, 4 replicates. Relative quantitation of the mRNA is determined by the difference in threshhold cycles between the internal control and

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the gene of interest (see Example 12 below). In an embodiment of the invention, quantitative PCR is performed using amplification, reporting agents and instruments such as those supplied commercially by Applied Biosystems (Foster City, CA). Given a defined efficiency of amplification of target transcripts, the point (e.g., cycle number) that signal from amplified target template is detectable may be directly related to the amount of specific message transcript in the measured sample. Similarly, other quantifiable signals such as fluorescence, enzyme activity, disintegrations per minute, absorbance, etc., when correlated to a known concentration of target templates (e.g., a reference standard curve) or normalized to a standard with limited variability can be used to quantify the number of target templates in an unknown sample.

Page 45, first paragraph Please replace the first paragraph of page. 45 (lines 1-27) with the following version:

The specific primers are synthesized from data obtained from public databases (e.g., Unigene, National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD), including information from genomic and cDNA libraries obtained from humans and other animals. Primers are chosen to preferentially amplify from specific RNAs obtained from the test or indicator samples, see, for example, RT PCR, Chapter 15 in RNA Methodologies, A laboratory guide for isolation and characterization, 2nd edition, 1998, Robert E. Farrell, Jr., Ed., Academic Press; or Chapter 22 pp.143-151, RNA isolation and characterization protocols, Methods in molecular biology, Volume 86, 1998, R. Rapley and D. L. Manning Eds., Human Press, or ~~14~~ in Statistical refinement of primer design parameters, Chapter 5, pp.55-72, PCR applications: protocols for functional genomics, M.A. Innis, D.H. Gelfand and J.J. Sninsky, Eds., 1999, Academic Press). Amplifications are carried out in either isothermic conditions or

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using a thermal cycler (for example, a ABI 9600 or 9700 or 7700 obtained from Applied Biosystems, Foster City, CA; see Nucleic acid detection methods, pp. 1-24, in Molecular methods for virus detection, D.L.Wiedbrauk and D.H., Farkas, Eds., 1995, Academic Press). Amplified nucleic acids are detected using fluorescent-tagged detection primers (see, for example, Taqman™ PCR Reagent Kit, Protocol, part number 402823 revision A, 1996, Applied Biosystems, Foster City CA.) that are identified and synthesized from publicly known databases as described for the amplification primers. In the present case, amplified DNA is detected and quantified using the ABI Prism 7700 Sequence Detection System obtained from ~~Appled~~ Applied Biosystems (Foster City, CA). Amounts of specific RNAs contained in the test sample or obtained from the indicator cell lines can be related to the relative quantity of fluorescence observed (see for example, Advances in quantitative PCR technology: 5' nuclease assays, Y.S. Lie and C.J. Petropolus, Current Opinion in Biotechnology, 1998, 9:43-48, or Rapid thermal cycling and PCR kinetics, pp. 211-229, chapter 14 in PCR applications: protocols for functional genomics, M.A. Innis, D.H. Gelfand and J.J. Sninsky, Eds., 1999, Academic Press.

Page 46, entire page Please replace p. 46 with the following corrected p. 46 as follows:

deoxyNTPs mixture, Random Hexamers, RNase Inhibitor, MultiScribe Reverse Transcriptase (50 U/mL) (2) RNase / DNase free water (DEPC Treated Water from Ambion (P/N 9915G), or equivalent)

Methods

1 ____Place RNase Inhibitor and MultiScribe Reverse Transcriptase on ice immediately. All other reagents can be thawed at room temperature and then placed on ice.

2 ____Remove RNA samples from –80~~o~~°C freezer and thaw at room temperature and then place immediately on ice.

3_____Prepare the following cocktail of Reverse Transcriptase Reagents for each 100 ~~m~~µL RT reaction (for multiple samples, prepare extra cocktail to allow for pipetting error):

	1 reaction(~~m~~µL)	11X, e.g. 10 samples(~~m~~µL)
10X RT Buffer	10.0	110.0
25 mM MgCl2	22.0	242.0
dNTPs	20.0	220.0
Random Hexamers	5.0	55.0
RNAse Inhibitor	2.0	22.0
Reverse Transcriptase	2.5	27.5
Water	18.5	203.5
Total:	80.0	880.0 (80 ~~m~~µL per sample)

4 Bring each RNA sample to a total volume of 20 ~~m~~µL in a 1.5 mL microcentrifuge tube (for example, for THP-1 RNA, remove 10 ~~m~~µL RNA and dilute to 20 ~~m~~µL with RNase / DNase free water…for whole blood RNA use 20 ~~m~~µL total RNA) and add 80 ~~m~~µL RT reaction mix from step 5.2.3. Mix by pipetting up and down.

5 Incubate sample at room temperature for 10 minutes.

6 Incubate sample at 37~~o~~°C for 1 hour.

7 Incubate sample at 90~~o~~°C for 10 minutes.

8 Quick spin samples in microcentrifuge.

9 Place sample on ice if doing PCR immediately, otherwise store sample at –20~~o~~°C for future use.

10 PCR QC should be run on all RT samples using 18S and ~~b~~β-actin (see SOP 200-020).

Example 2. Different inflammatory stimuli give rise to different, baseline

Page 49 through page 50, Example 5 Please replace the text of Example 5, which spans p. 49, line 25 – p. 50, line 12, with the following text:

Example 5. Similarities and differences in the effect of a single agent on cell populations differing in their biological condition.

Ex-vivo gene expression analysis can be performed by obtaining the blood of a subject for example by drawing the blood into a vacutainer tube with sodium heparin as an anticoagulant. An anti-inflammatory such as 3-methyl-prednisolone at a final concentration of 10 micromolar was added to blood in a polypropylene tube, incubated for 30 minutes at 37°C in 5% CO_2 . After 30 minutes a stimuli such as LPS at 10 ng/mL or heat killed staphlococcus (HKS) at 1:100 dilution was added to the drug treated whole blood. Incubation continued at 37°C in 5% CO_2 for 6 hours unless otherwise indicated. Erythrocytes were lysed in RBC lysis solution (Ambion) and remaining cells were lysed according to the Ambion RNAqueous-Blood module (catalog # 1913). RNA was eluted in Ambion elution solution. RNA was DNAsed treated with 1 unit of DNAse I (Ambion #2222) in 1X DNAse buffer at 37°C for 30 minutes. In this example, first strand synthesis was performed using the Applied Biosystems TaqMan Reverse Transcriptase kit with MultiScribe reverse transcriptase (catalog # N808-0234). Quality check of RT reactions were performed with Taqman PCR chemistry using the 18S rRNA pre-developed assay reagents (PDAR) from Applied Biosystems (part #4310893E). PCR assay of Source Selected Profiles were performed on 6 to 24 genes in four replicates on the Applied Biosystems 7700. PCR assays were performed according to specifications outlined with the PDAR product. Relative quantitation of the gene of interest was calibrated against 18S rRNA expression as described in Applied Biosystems product User Bulletin 2 (1997) and elaborated in Hirayama, et al (Blood 92, 1998:46-52) using 18S instead of GAPDH. Relative quantitation of the mRNA was measured by the difference in threshold cycles between 18S and the gene of interest. This delta C_T was then compared to the normalizing condition, either subject before treatment, or stimuli without drug in an *ex-vivo* assay to measure "fold induction" represented in the bar graphs (Figure 14). For example, in the above graph, IFN- levels are 1/50 less on day 3 than before treatment.

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Page 51 through page 52, Example 8 Please replace the text of Example 8, which spans p. 51, line 20 through p. 52, line2, with the following text as follows:

Example 8. A calibrated profile data set may provide a signature profile for a complex mixture of compounds.

Figure 21 illustrates the effect of three different anti-inflammatory herbs on a selected panel of constituents including constituents of an Inflammation Selected Panel (TNF-~~a~~α, Il-1b, ICAM, Il-8, Il-10, Il-12p40, ICE, cox-2, cox-1 and mmp-3) a cell growth and differentiation selected panel (c-fos, c-jun and STAT3) , a toxicity selected panel (SOD-1, TACE, GR, HSP70, GST, c-fos, c-jun, INOS) and a liver metabolism selected panel (INOS, cyp-a and u-pa). The cells assayed in Figure 21 are aliquots of blood from a subject that are exposed *ex vivo* to lipopolysaccharide and to Echinacea (SPM9910214) Arnica (SPM9910076) and Siberian Ginseng (SPM9910074), each of the nutraceuticals being applied to the blood sample at the same concentration of 200ug/ml. The baseline is cell sample with lipopolysaccharide in the absence of a nutraceutical. Each nutraceutical (formed from a complex mixture) has a characteristic signature profile just as did the single compound pharmaceutical anti-inflammatory agents. The signature profile may be provided in a graphic form that can be use to identify a herbal while providing information concerning its properties and its efficacy for a single subject or for an average population of subjects.

Page 56 through page 57, Examples 16 and 17 Please replace the text of Examples 16 and 17 with the following text for these examples as follows:

Example 16. The effect of different agents is evaluated using a subset of the Selected Prostate Panel.

In Figure 30 is shown the response of ~~a subject [or in vitro?]~~ five different cell lines to the

administration of various agents, using a subset of the Selected Prostate Panel (listed in Table 5). ~~[details]~~ This figure also shows broad functions of constituents of the panel.

Example 17. The use of a rat liver metabolism selected panel to measure the effect of a pharmaceutical agent, clofibrate, Male rats were treated with 400mg/kg/day of clofibrate administered by mouth and the levels of gene expression were measured in liver tissue. Clofibrate is used here because its metabolism in the rat and human liver is well described. As expected, clofibrate induces gene expression at the cyp 1A1 locus, but the agent also induces expression at a number of other metabolic loci in the selected panel as measured in this cohort of in-bred Spraque-Dawley rats. The ratio of the concentration of each constituent for the clofibrate treated rats is measured with reference to a control (baseline) which is a set of rats treated only with the carrier compound. The resultant selected profile is provided in Figure 31.